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Utilization of Antidrug Antibody Fragments for the Optimization of Intraperitoneal Drug Therapy: Studies Using Digoxin as a Model Drug

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ABSTRACT

The direct administration of chemotherapeutic agents into the peritoneal cavity has been investigated as a method to treat cancers residing within the peritoneum. The benefits of i.p. drug administration are limited, however, by the systemic toxicity of antineoplastic drugs which diffuse out of the peritoneum and into the general circulation. We propose that antidrug antibody fragments may be useful in binding chemotherapeutics in the general circulation, thereby reducing the systemic tissue exposure and toxicity resulting from such i.p. therapy. Inasmuch as antibody fragments directed against antineoplastic agents are not available, we tested our hypothesis by using i.v. administered ovine antidigoxin Fab fragments and determined their ability to limit digoxin tissue exposure and toxicity in mice after an i.p. digoxin injection. The rate of digoxin disappearance from the peritoneal cavity and the fraction of digoxin unbound in the peritoneal cavity were also assessed to determine the effect of the antibody fragments on peritoneal exposure. Our results showed that the antidigoxin antibody fragments can greatly decrease digoxin tissue exposure and toxicity without affecting peritoneal exposure, unbound fraction of digoxin in the peritoneum or peritoneal digoxin disappearance rate. Although the utility of drug-binding antibodies and antibody fragments for the treatment of drug intoxication is well known, these results demonstrated the potential ability of antidrug antibody fragments to improve the site-specificity of drug therapy.

Direct drug delivery into the peritoneal compartment has been studied as a method to treat cancers residing within the peritoneum. It has been theorized that i.p. administration of cancer chemotherapeutic agents would allow for greater drug exposure to the tumor while simultaneously decreasing systemic exposure and toxicity (Dedrick *et al.*, 1978; Clay and Howell, 1992). Unfortunately, results of early phase I and phase II clinical studies assessing the benefits of i.p. delivery have shown lower than optimal decreases in toxicity or increases in effect (Howell, 1988; Clay and Howell, 1992).

One reason for the lack of success of direct i.p. therapy for some drugs is the development of toxicity arising from the distribution of the drug from the peritoneal cavity into the general circulation. An approach to overcome this problem is to administer drug into the peritoneum and simultaneously a drug-complexing agent into the systemic circulation, so as to reduce the exposure of systemic tissues to free drug. Howell *et al.* (1982) have investigated this optimization approach in a study of the utility of sodium thiosulfate for the reduction of cisplatin toxicity. The benefit of this combined therapy, however, was limited by the slow rate of thiosulfate-cisplatin complexation in plasma (Howell, 1988).

We postulate that antidrug antibody fragments may be superior complexing agents for this approach. Antibody fragments may be produced against a wide variety of substances, including many drugs. Antibodies generally exhibit extremely high specificity and binding affinity for their antigen (K_{eq} common range: 10^4 to 10^{10} M^{-1}), as well as rapid binding rates (commonly: 10^8 $M^{-1} \cdot sec^{-1}$) (Stryer, 1988). In addition, the high molecular weight and polar characteristic of antibody fragments should result in favorable distribution properties (*i.e.*, the antibodies are expected to remain primarily in plasma, with a very slow diffusion rate across the peritoneal membrane).

To determine the potential use of antidrug antibody fragments for the optimization of i.p. drug delivery, we have conducted a preliminary investigation into the general pharmacokinetic and physiological implications of the approach, by using the cardiac glycoside digoxin and ovine antidigoxin Fab antibody fragments (Digibind, Burroughs-Wellcome Company, Re-

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ABBREVIATIONS: AUC_T, area under the concentration *vs.* time curve; AUC_P, area under the peritoneal concentration *vs.* time curve; FE_T, fractional tissue exposure; FE_P, fractional peritoneal exposure; FRIA, fluorescence polarization immunoassay; Fu(p), fraction of digoxin unbound in the peritoneal cavity.

search Triangle Park, NC) as model agents. Use of a model system was necessitated because antibody fragments directed against commonly used cancer chemotherapeutic agents are not yet available.

The present study was designed, therefore, to determine whether the simultaneous administration of digoxin (i.p.) and antidigoxin antibody fragments (i.v.) would: 1) reduce observable digoxin toxicity; 2) reduce systemic digoxin tissue exposure; 3) affect the unbound fraction of digoxin within the peritoneal cavity; and 4) affect the disappearance rate of digoxin from the peritoneal cavity, when compared to administration of digoxin (i.p.) alone.

In addition, computer simulations were conducted to predict the ability of the antibody fragments to affect digoxin tissue and peritoneal exposure. The results of these simulations were used to determine an appropriate antibody fragment dose to minimize tissue exposure while not affecting peritoneal exposure. Further simulations were conducted to assess the influence of the antibody fragment-digoxin association rate, dissociation rate and affinity on tissue exposure. Simulations were carried out by using the model depicted in figure 1 through the use of ADAPT II pharmacokinetic software (D'Argenio and Schumitzky, 1992).

Methods

Simulations. The pharmacokinetic model used to describe digoxin disposition in the presence of drug binding antibody fragments is shown as figure 1 and is based on the following equations:

$$\frac{dC_{dP}}{dt} = -(k_a \cdot C_{dP}) - (k_{on} \cdot C_{aP} \cdot C_{dP}) + (k_{off} \cdot C_{daP}) \quad (1)$$

$$\frac{dC_{aP}}{dt} = \frac{CL_{Da} \cdot C_{aB}}{V_P} - \frac{CL_{Da} \cdot C_{aP}}{V_P} - (k_{on} \cdot C_{aP} \cdot C_{dP}) + (k_{off} \cdot C_{daP}) \quad (2)$$

$$\frac{dC_{daP}}{dt} = \frac{CL_{Da} \cdot C_{daB}}{V_P} - \frac{CL_{Da} \cdot C_{daP}}{V_P} + (k_{on} \cdot C_{aP} \cdot C_{dP}) - (k_{off} \cdot C_{daP}) \quad (3)$$

$$\frac{dC_{dB}}{dt} = \frac{k_a \cdot C_{dP} \cdot V_P}{V_{dC}} + \frac{CL_{Dd} \cdot C_{dT}}{V_{dC}} - \frac{CL_{Dd} \cdot C_{dB}}{V_{dC}} - (k_{el} \cdot C_{dB}) - (k_{on} \cdot C_{dB} \cdot C_{aB}) + \frac{k_{off} \cdot C_{daB} \cdot V_a}{V_{dC}} \quad (4)$$

$$\frac{dC_{aB}}{dt} = -\frac{CL_{Da} \cdot C_{aB}}{V_a} + \frac{CL_{Da} \cdot C_{aP}}{V_a} - (k_{el,2} \cdot C_{aB}) - \frac{k_{on} \cdot C_{dB} \cdot C_{aB} \cdot V_{dC}}{V_a} + (k_{off} \cdot C_{daB}) \quad (5)$$

$$\frac{dC_{daB}}{dt} = -\frac{CL_{Da} \cdot C_{daB}}{V_a} + \frac{CL_{Da} \cdot C_{daP}}{V_a} - (k_{el,2} \cdot C_{daB}) + \frac{k_{on} \cdot C_{dB} \cdot C_{aB} \cdot V_{dC}}{V_a} - (k_{off} \cdot C_{daB}) \quad (6)$$

$$\frac{dC_{dT}}{dt} = \frac{CL_{Dd} \cdot C_{dB}}{V_{dT}} - \frac{CL_{Dd} \cdot C_{dT}}{V_{dT}} \quad (7)$$

Where C_{dP}, C_{aP} and C_{daP} are the concentrations of digoxin, free antibody fragments and antibody fragment-digoxin complex in the peritoneal cavity; C_{dB}, C_{aB} and C_{daB} are the concentrations of digoxin, free antibody fragment and antibody fragment-digoxin complex in their respective central distribution volumes; C_{dT} is the concentration of digoxin within the tissue compartment and t is time. Also, k_a, k_{on}, k_{off}, k_{el} and $k_{el,2}$ are the rate constants for digoxin absorption from the peritoneum, antibody fragment-digoxin association, antibody fragment-digoxin dissociation, elimination of digoxin and elimination of antibody fragments, respectively. CL_{Dd} is the distribution clearance of digoxin; and CL_{Da} is the distribution clearance of antibody fragments between the peritoneal and central compartments. V_a, V_{dC}, V_{dT} and V_P are the volumes of distribution of the antibody fragments, the central volume of distribution of digoxin, the tissue volume of distribution for digoxin and the volume of the peritoneal cavity, respectively.

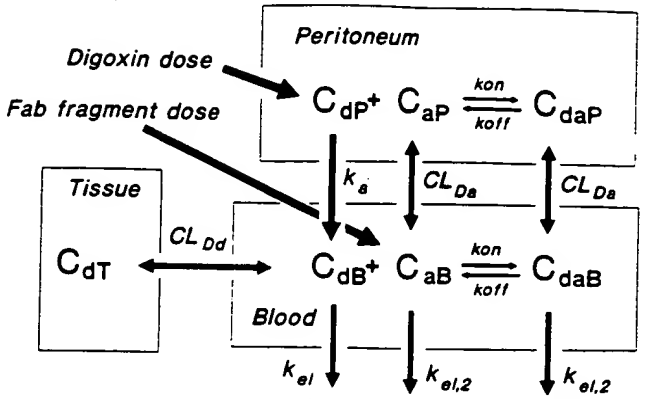

Fig. 1. Pharmacokinetic model used for simulation of the disposition of digoxin and antidigoxin antibody fragments in the mouse. Parameters are described within the text.

Digoxin pharmacokinetic parameters for the mouse (CL_{Dd}, k_{el}, V_{dC} and V_{dT}) were calculated by conversion of published biexponential data (Griffiths *et al.*, 1984) to data consistent with a two-compartment model. The rate constant k_a was determined experimentally in preliminary experiments. Antidigoxin Fab fragment parameters (V_a and $k_{el,2}$) were also taken from Griffiths *et al.* (1984). The elimination and distribution of Fab fragments and the Fab fragment-digoxin complex were assumed to be identical. CL_{Da} was estimated to be 9×10^{-7} liters kg^{-1} min^{-1}.

A series of simulations was conducted to aid in the determination of an appropriate antibody fragment dose for the animal study. In this exercise, the dose of digoxin administered was held at 1.02×10^{-6} mol/kg whereas the antibody fragment dose was increased from 10^{-10} mol/kg to 10^{-5} mol/kg. The association and dissociation rate constants for the antibody fragment were fixed in this series at: $k_{on} = 10^8$ $M^{-1} \cdot sec^{-1}$ and $k_{off} = 10^{-1} \cdot sec^{-1}$; producing an equilibrium constant consistent with published values for the antidigoxin Fab fragments (K_{eq}: 10^9 M^{-1}, Smith *et al.*, 1970; Curd *et al.*, 1971). The AUC_T and the AUC_P were obtained *via* the linear trapezoidal method from 0 to 240 min at each dose of the antibody. The FE_T after antibody fragment administration was defined as the AUC_T obtained at each antibody fragment dose divided by the AUC_T observed when digoxin was given without antibody fragment treatment. The FE_P was defined in the same manner.

In a second series of simulations, the effect of altering the antibody fragment association rate, dissociation rate and affinity on FE_T were assessed. The binding parameters were tested in the following ranges: k_{on}, 10^1 to 10^8 M^{-1} sec^{-1}; k_{off}, 10^{-1} to 10^6 sec^{-1}; K_{eq}, 10^2 to 10^9 M^{-1}. For these simulations, the doses of antibody fragments and digoxin were held at 1.02×10^{-6} mol/kg. These simulations were conducted to determine which complexation parameter was most important for reducing tissue exposure.

Mortality experiment. Male Balb-C mice (weights: 12.5–23 g; Harlan, Indianapolis, IN) were paired into two weight matched groups ($n = 10$ animals/group). All animals received 8 mg/kg of digoxin (lot no. 03223; Elkins-Sinn, Cherry Hill, NJ) administered by i.p. injection. In the test group of mice, an equimolar dose of antidigoxin ovine immune Fab fragments (512 mg/kg, Digibind, lot no. 8X2748; Burroughs Wellcome) was administered by i.v. injection (*via* the tail vein) 3 to 5 min before the digoxin dose. Animals were monitored for cessation of respiration and survival time was recorded. The experiment was terminated at 240 min after the digoxin injection and surviving animals were sacrificed by cervical dislocation.

The digoxin injection solution contained 250 μg/ml of digoxin,

solubilized in 40% propylene glycol and 10% ethanol. The antidigoxin Fab fragment injection solution contained 40 mg/ml of Fab fragments in distilled water.

Tissue exposure. Male Balb-C mice were randomly separated into a control group (animals receiving i.p. digoxin alone, 800 μg/kg, as a digoxin solution of 25 μg/ml in 4% propylene glycol and 1% ethanol) and a test group (animals receiving i.p. digoxin, 800 μg/kg, and i.v. antidigoxin antibody fragments, 64 mg/kg, as a 10 mg/ml solution in distilled water). The antibody fragment dose used was approximately equimolar to the dose of digoxin administered, assuming a specific binding activity of 80% and a MW of 50,000 daltons for the antidigoxin Fab fragment (Curd *et al.*, 1971; Cano *et al.*, 1992). Three animals from each group were sacrificed by cervical dislocation at 5, 15, 30 and 60 min after injection of digoxin.

Immediately after sacrifice, 5 ml of normal saline was injected into the peritoneal cavity of the expired animal. After massaging the abdomen of the animal, 1 ml of fluid was removed from the peritoneum for analysis of free and total digoxin concentration. The total amount of digoxin in the peritoneal cavity was calculated, assuming a total final peritoneal fluid volume of 5 ml (*i.e.*, it was assumed that the volume added to the peritoneal cavity after sacrifice was very much larger than the peritoneal fluid volume just before sacrifice). The heart, brain and left hind-leg skeletal muscle were also collected for analysis. From these data, the fraction of unbound digoxin in the peritoneum, peritoneal disappearance rate of digoxin and the AUC_Ts were obtained. The AUC_Ts were obtained from 0 to 60 min *via* the linear trapezoidal method, by using the mean of the three tissue concentrations at each sacrifice time.

Tissue extraction. The method used for extraction of digoxin was modified from that of Berman *et al.* (1977) for the extraction of digoxin from sheep tissues. Briefly, tissues removed from treated animals were weighed wet, minced with a razor blade, then suspended in 6 ml of normal saline through the use of a Polytron tissue homogenizer (model PT10/35). Digoxin was extracted from the tissue suspension with two washes of 10 ml of methylene chloride. The pooled methylene chloride phases were evaporated to dryness under a stream of nitrogen gas. The extracted digoxin was then reconstituted with normal saline for analysis. The reconstitution volume was varied to prepare samples within the range of analysis specified by the Abbott TDx, *i.e.*, 0.2 to 5 ng/ml.

The efficiency and variability of this method were determined by assessing the extraction of known quantities of digoxin which had been added to prepared tissue homogenates. Five homogenates of mouse heart, skeletal muscle and brain were prepared as above. Digoxin was added directly into the homogenates, which were then treated as described above.

Similarly, the efficiency and variability of extraction of digoxin from the antibody fragments were assessed. Digoxin and antidigoxin Fab fragments were added to a reaction vial in sufficient quantities to produce measurable amounts of total and free digoxin. The contents of the vial were incubated for 90 min at 4°C. Aliquots of 2 ml were removed and subjected to extraction as described above.

Digoxin assay. Digoxin concentrations were analyzed by FPIA with the use of an Abbott TDx automated FPIA device (Digoxin II kits, lot nos. 64094Q100 and 66519Q100: Abbott Laboratories, Abbott Park, IL). As the assay kit was calibrated with human serum standards, a standard curve was constructed with samples in normal saline to avoid inaccuracy due to matrix differences. Correction for matrix differences was accomplished through the use of the following equation obtained from the standard curve: $[digoxin]_{true} = 0.847 \cdot [digoxin]_{assayed} + 0.14$ ng/ml. FPIA was chosen as the assay method of choice because of its ability to provide accurate total digoxin concentrations in spite of the presence of low levels of therapeutic antibody fragments (Hansell, 1989; Argyle, 1986).

Ultrafiltration. The free concentration of digoxin in the peritoneal fluid was determined through the use of Centrifree ultrafiltration tubes (lot no. MCA129; Amicon, Beverly, MA). Ultrafiltrated samples were centrifuged at 4000 rpm (approximately 1200 × *g*) for 15 min. A standard curve was generated for assessment of ultrafiltrated samples, as ultrafiltration itself may affect the reported digoxin concentration (Banner *et al.*, 1992). The equation obtained from the standard curve to correct for the combined effect of the normal saline matrix and ultrafiltration was: $[digoxin]_{true} = 0.893 \cdot [digoxin]_{assayed} + 0.18$ ng/ml. Ultrafiltration of digoxin by this method was shown previously to provide accurate free digoxin concentrations (Hursting *et al.*, 1987).

Statistics. Data are presented as mean ± S.D. Significant differences were concluded if $P < .05$, as determined by Student's *t* tests, comparing the control (digoxin alone) *vs.* the antibody fragment group (digoxin and antibody fragments administered).

Results

Simulations. The dose-ranging simulations conducted produced a sigmoidal log antibody fragment *vs.* FE_T curve. As expected, the FE_T (the ratio of the digoxin AUC_T obtained after Fab fragment administration relative to control) decreased as the antibody fragment dose was raised relative to the digoxin dose. However, the FE_P remained unchanged in the antibody fragment dose range examined (10^{-10} to 10^{-5} mol/kg, fig. 2). These simulations therefore predicted that dramatic reductions in tissue exposure may be produced at antibody fragment doses which do not decrease peritoneal exposure. At the antibody fragment dose used for the tissue exposure study, 1.02×10^{-6} mol/kg, the simulation produced an AUC_T which was 14.1% of control and an AUC_P which was 100% of control.

The second series of simulations demonstrated that the antibody fragment-digoxin equilibration constant is the binding parameter related most closely to antibody fragment efficacy. FE_T was shown to decrease with increasing antibody fragment affinity (fig. 3); however, paired changes in the association and dissociation rate constants (*i.e.*, k_{on} and k_{off} were altered whereas K_{eq} was held constant) did not affect fractional tissue exposure (data not shown).

Extraction efficiency. The extraction of digoxin from tissues and antidigoxin Fab fragments was of good efficiency and low variability in all cases. Efficiencies for the mouse heart,

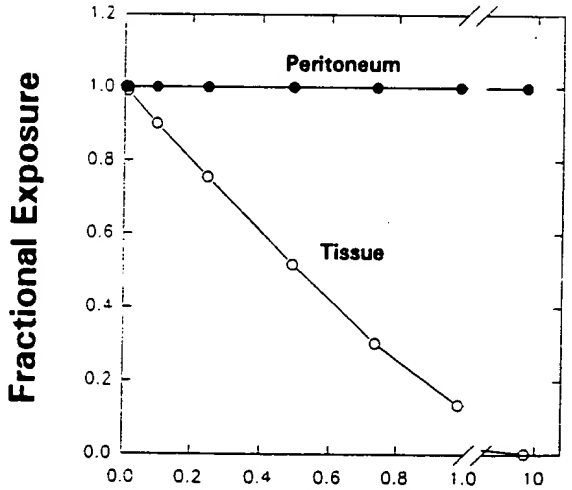

Fig. 2. Simulated fractional exposure of digoxin in the peritoneal (●) and tissue (○) compartments of the mouse, as a function of antibody fragment/digoxin dose ratio. Fractional exposure after antibody fragment administration is obtained by dividing the AUC (0–240 min) at each antibody fragment dose by the AUC (0–240 min) observed when no antibody fragments were given. Th d se of digoxin used in this simulation was held at 1.02×10^{-6} mol/kg, whereas the antibody fragment dose was increased from 10^{-11} t 10^{-5} mol/kg. Th values of the model parameters used in these simulations were held constant as described in th text.

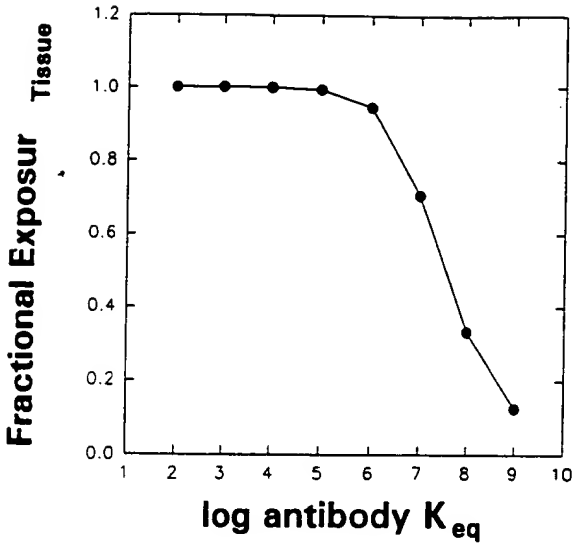

Fig. 3. The simulated relationship of fractional tissue exposure and antibody fragment affinity. The doses of digoxin (i.p.) and antidigoxin Fab fragments (i.v.) were each held at 1.02×10^{-6} M/kg. The range of k_{on} used in these simulations was: 10 to 10^8 M^{-1} sec^{-1}, whereas the range for k_{off} was: 10^{-1} to 10^5 sec^{-1}. Fractional tissue exposure after Fab fragment administration was obtained by dividing the AUC_T (0–240 min) at each Fab fragment dose by the AUC_T (0–240 min) observed when no antibody fragments were given.

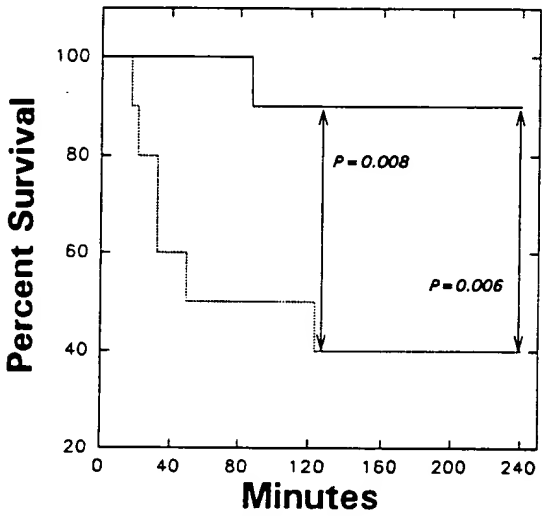

Fig. 4. The percentage of animals surviving a toxic dose of digoxin (8 mg/kg) is shown for animals receiving digoxin alone (···) and those receiving digoxin i.p. and antidigoxin antibody fragments (512 mg/kg) i.v. (——). Mean survival time was used for statistical comparison of the two groups.

skeletal muscle, brain tissue and from the antibody fragments were: 87.0 ± 8.6, 73.2 ± 4.5, 74.7 ± 5.4 and 93.7 ± 1.1%, respectively. Tissue concentrations reported were corrected for these extraction efficiencies.

Mortality. Administration of the antidigoxin fragments greatly increased survival rate. At 240 min after digoxin injection, the survival rate for the antibody group was 90% whereas only 40% of the control group (digoxin alone) survived ($P = .006$). Pretreatment with the antibody fragments decreased mortality substantially (fig. 4).

Tissue exposur . In the three tissues monitored (heart, skeletal muscle and brain), significant reductions in digoxin concentrations were observed (table 1). The AUC_T was reduced substantially in the antibody fragment-treated group relative to the control group. The FE_T, defined as the AUC_T Fab/AUC_T control, was 0.56, 0.17 and 0.71 for the heart, muscle and brain, respectively.

Fraction unbound. The Fu(p) of the antibody fragment-treated group was not found to be statistically different from the control group at any time (table 1). This finding is supportive of the expected slow rate of diffusion into the peritoneal cavity for the antibody Fab fragment.

Disappearance rate. The rate of disappearance of peritoneal digoxin, which was calculated from the decrease of total peritoneal digoxin with time, was not significantly different for the two groups ($T_{½}$ absorption was 12.0 ± 2.0 min for the Fab fragment group and 9.8 ± 1.8 min for the control group). The presence of antibody fragments in the systemic circulation did not appear to influence the rate of digoxin diffusion from the peritoneal cavity. This finding was anticipated because the concentration of digoxin in the peritoneal cavity was expected to be much greater than the free concentration of digoxin in plasma until very late in the experiment when a significant fraction of digoxin has been absorbed. A "sink" condition existed for both the antibody fragment and control groups; therefore, the disappearance rate of peritoneal digoxin was largely unaffected.

The peritoneal exposure to digoxin, which is a function of the disappearance rate of digoxin from the peritoneum and of the Fu(p), was not different for the Fab fragment group compared to the control group.

Discussion

Howell *et al.* (1982) investigated the utility of a competitive binding agent (sodium thiosulfate) for the reduction of the systemic toxicity of an i.p. cisplatin infusion. The investigators simultaneously infused sodium thiosulfate (i.v.) and cisplatin (i.p.) into several ovarian cancer patients, with the expectation that the sodium thiosulfate would bind and neutralize cisplatin immediately as it entered the bloodstream, thereby preventing systemic exposure and toxicity. Unfortunately, it was found that sodium thiosulfate was not able to increase the plasma clearance of free cisplatin significantly, nor was the binding agent able to greatly decrease systemic exposure to cisplatin substantially (Howell *et al.*, 1982). This failure was presumably due to the slow rate of thiosulfate-cisplatin complexation ($T_{½}$ = 225 min) relative to the elimination rate of free cisplatin by other means ($T_{½}$ = 66 min) (Howell, 1988).

The present study suggests that antidrug antibody fragments may be superior complexing agents for the optimization of i.p. drug delivery. Antibody fragments generally exhibit rapid binding rates, high specificity and affinity, as well as desirable distribution and elimination characteristics. We have investigated the potential use of antibody fragments for the optimization of i.p. drug delivery through a series of experiments and computer simulations. Digoxin and antidigoxin antibody fragments were used as model agents for testing this approach.

Our experimental results demonstrate that antibody fragments may decrease the systemic exposure and toxicity of digoxin without affecting the peritoneal exposure, fraction unbound or disappearance rate. Systemic exposure and toxicity, therefore, have been dissociated from local drug exposure through the use of drug binding antibodies. These results suggest that drug toxicity may be prevented without affecting drug exposure at the active site.

TABLE 1

Digoxin tissue concentrations and Fu(p) for the antibody and control groups

Values are mean ± S.D.

	Time							
	5 min		15 min		30 min		60 min	
	Fab	Control	Fab	Control	Fab	Control	Fab	Control
	Tissue Concen. of Digoxin (ng/g [wet tissue wt.])							
Heart	24.8 ± 4.6**	77.5 ± 15.8	170.7 ± 93.4	277.0 ± 99.4	199.2 ± 44.5**	337.7 ± 34.3	137.2 ± 63.6*	286.0 ± 50.8
Muscle	6.1 ± 1.8*	24.3 ± 10.5	64.8 ± 67.8*	159.8 ± 23.6	30.2 ± 20.7***	238.6 ± 19.9	48.3 ± 52.6***	409.3 ± 21.0
Brain	3.9 ± 0.5**	10.2 ± 2.3	20.0 ± 5.0	15.2 ± 6.3	15.5 ± 1.3*	22.7 ± 4.4	11.5 ± 2.4***	23.7 ± 1.7
Fu(p)	0.80 ± 0.01	0.73 ± 0.004	0.83 ± 0.03	0.89 ± 0.19	0.86 ± 0.44	0.78 ± 0.16	0.65 ± 0.08	0.70 ± 0.16

* $P < 0.05$ compared to the control group; ** $P < 0.01$ compared to the control group; *** $P < 0.001$ compared to the control group.

The simulations conducted assumed a small value for the distribution clearance of Fab fragments between the peritoneal and central compartments. Unfortunately, we were unable to measure the rate of appearance of Fab in the peritoneal compartment to obtain an experimental estimate for this clearance value. However, the use of a small value was consistent with the experimental observation that the Fu(p) and the rate of digoxin disappearance from the peritoneal cavity were not different in the Fab fragment-treated group relative to the control group. If the distribution clearance of the Fab fragments had a larger value, then the unbound fraction of drug in the peritoneum and the rate of digoxin exiting the peritoneum would be expected to be altered.

The observed experimental results were predicted qualitatively through computer simulation. The use of a "physiological" model (Gerlowski and Jain, 1983) may allow for more accurate estimation of individual tissue exposures than the primitive "tissue cluster" model which was used for our simulations. However, the practical utility of such a model may be limited by its complexity. The model which we have presented may have general utility in describing drug disposition in the presence of binding substances. This model may be useful in screening drug candidates for the proposed antibody complexation approach, as well as for the development of optimal dosing strategies.

Although the utility of drug binding antibodies for the treatment of drug toxicity has been well documented (Smith *et al.*, 1976; Antman *et al.*, 1990; Brunn *et al.*, 1992), our results have demonstrated the potential utility of antidrug antibody fragments for the enhancement of drug site-specificity. It is possible that the proposed approach may be used as an adjunct in the treatment of cancers contained within the peritoneum. Systemic toxicity, particularly bone marrow depression, is often the dose-limiting factor in cancer chemotherapy. Optimization of i.p. drug delivery through antibody complexation may substantially shift the toxicity *vs.* dose curves of antineoplastic drugs administered into the peritoneum, thus allowing more drug to be given at acceptable levels of toxicity. Cure rates can be expected to follow the increase in administered dose, as most cancer chemotherapeutic agents are known to have a steep dose *vs.* response relationships.

There are, however, several concerns which must be addressed before the implementation of this type of therapy. First, the alteration of drug distribution which accompanies antibody-drug complexation may result in a potentiation of drug toxicities or the development of new drug toxiciti s in certain cases. For example, Faulstich *et al.* (1988) have shown an nhancement of the renal toxicity of α-amantin (a mushroom toxin) when this toxin was coadministered with antiamantin immunoglobulin G and Fab fragments. This toxicity is presumed to result from an increased delivery of the toxin (as the antibody-toxin complex) to protein-metabolizing cells of the kidney. The risk of redistributing systemic toxicity, rather than minimizing systemic toxicity, should be appreciated as a potential outcome of the proposed approach.

Additional important concerns are: 1) production and purification of antibodies directed against antineoplastic agents; 2) immunogenicity of the antibodies; and 3) cost of therapy. Further work on overcoming these formidable hurdles is required before the practicality of the proposed approach may be validated fully.

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